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Cannabinoid physiology and pharmacology: 30 years of progress

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Abstract

Δ^9-Tetrahydrocannabinol from *Cannabis sativa* is mimicked by cannabimimetic analogs such as CP55940 and WIN55212-2, and antagonized by rimonabant and SR144528, through G-protein-coupled receptors, CB_1 in the brain, and CB_2 in the immune system. Eicosanoids anandamide and 2-arachidonoylglycerol are the "endocannabinoid" agonists for these receptors. CB_1 receptors are abundant in basal ganglia, hippocampus and cerebellum, and their functional activity can be mapped during behaviors using cerebral metabolism as the neuroimaging tool. CB_1 receptors couple to $G_{i/o}$ to inhibit cAMP production, decrease Ca^{2+} conductance, increase K^+ conductance, and increase mitogen-activated protein kinase activity. Functional activation of G-proteins can be imaged by [35S]GTPγS autoradiography. Post-synaptically generated endocannabinoids form the basis of a retrograde signaling mechanism referred to as depolarization-induced suppression of inhibition (DSI) or excitation (DSE). Under circumstances of sufficient intracellular Ca^{2+} (e.g., burst activity in seizures), synthesis of endocannabinoids releases a diffusible retrograde messenger to stimulate presynaptic CB_1 receptors. This results in suppression of γ-aminobutyric acid (GABA) release, thereby relieving the post-synaptic inhibition. Tolerance develops as neurons adjust both receptor number and cellular signal transduction to the chronic administration of cannabinoid drugs. Future therapeutic drug design can progress based upon our current understanding of the physiology and pharmacology of CB_1, CB_2 and related receptors. One very important role for CB_1 antagonists will be in the treatment of craving in the disease of substance abuse.

Keywords: Adenylyl cyclase; Anandamide; Antinociception; 2-Arachidonoylglycerol; Basal ganglia; Cognition; CP55940; Depolarization-induced suppression of inhibition (DSI); Dronabinol; Endocannabinoid; G-protein-coupled receptors; Hippocampus; Limbic system; Memory; Rimonabant; SR141716; Striatum; Δ9-Tetrahydrocannabinol; Voltage controlled Ca^{2+} channels; WIN55212-2

1. Cannabinoid pharmacology

1.1. Plant-derived and synthetic cannabimimetic agents

Ingestion of *Cannabis sativa* preparations such as marijuana (leaves and flowering tops) or ganja (resin) results in an intoxication characterized by sedation, cognitive dysfunction, failure to consolidate short-term memory, alteration in time assessment, perceptual changes, motor incoordination and poor executive function (see Abood and Martin, 1992; Dewey, 1986; Hollister, 1986; Pertwee, 1988 for review). Cannabinoid compounds isolated from the plant *C. sativa* comprise a family of tricyclic ring structures characterized by a phenol ring having a 5-carbon alkyl chain meta to the hydroxyl, a central pyran ring, and a mono-unsaturated cyclohexyl ring (Fig. 1) (see Mechoulam, 1970; Agurell et al., 1986; Howlett et al., 2002 for review). Cannabinoid receptor pharmacology as we now know it began 40 years ago when Δ^9-tetrahydrocannabinol (Δ^9-THC) (Fig. 1) was isolated and synthesized by Mechoulam's laboratory, and demonstrated to be the primary psychoactive constituent of marijuana (Mechoulam et al., 1967; Mechoulam and Gaoni, 1967). Other cannabinoid compounds, including cannabinol and cannabidiol, fail

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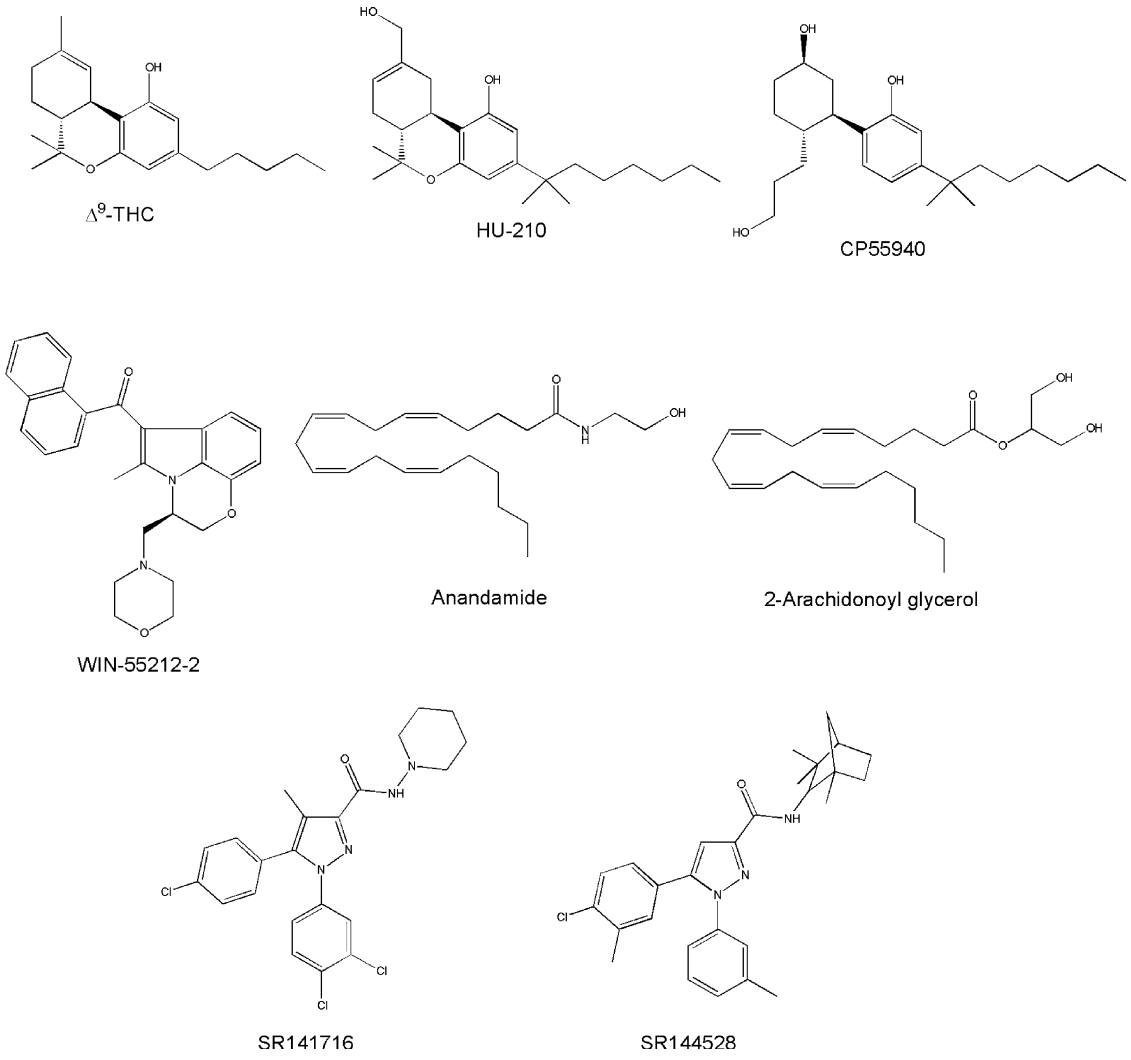

Fig. 1. Stuctures of cannabinoid receptor agonists, antagonists, and endogenous agonists.

to elicit the same psychoactive effects as Δ^9-THC, but can exhibit anticonvulsant activity and induce hepatic metabolic enzymes (Agurell et al., 1986; Hollister, 1986; Consroe and Mechoulam, 1987; Bornheim et al., 1993; Bornheim and Correia, 1989). An abundant series of cannabinoid analogs have been synthesized and tested in animal models for behaviors that include motor dysfunction and hypoactivity, immobility, hypothermia, antinociception, and deficits in memory and cognition (see Martin, 1986; Razdan, 1986; Compton et al., 1993; Pertwee, 1999; Howlett et al., 1995, 2002 for review). These effects have been attributed to activation of the CB_1 cannabinoid receptor, a G-protein-coupled receptor found abundantly in brain. Effects of Δ^9-THC on immune function have been attributed to the CB_2 cannabinoid receptor, also coupled to G-proteins, found predominantly in immune cells (Howlett et al., 2002). Synthetic Δ^9-THC (dronabinol) is approved for use in the USA to curtail nausea and vomiting in cancer chemotherapy, and to stimulate appetite in AIDS wasting syndromes (Mechoulam and Hanus, 2001; Pertwee, 2000).

Mammalian biotransformation of Δ^9-THC results in poorly active or inactive derivatives that are hydroxylated along the alkyl side chain and on the cyclohexyl ring (Agurell et al., 1986). However, hydroxylation at the 11-methyl group extending from the cyclohexyl ring results in a metabolite having greater potency than Δ^9-THC. Replacement of the 11-methyl group with a hydroxyl yielded an analog having increased potency in animal tests of antinociception (Wilson and May, 1975). Addition of methyl groups to the C1′ of the alkyl side chain, and extension of the side chain length yielded HU210, a compound having much greater potency than Δ^9-THC (Howlett et al., 1990; Felder et al., 1995). A series of non-classical AC-bicyclic and ACD-tricyclic compounds developed as non-opioid analgesics by Pfizer, Inc. were never taken to market for the treatment

of pain (Johnson et al., 1981). However, certain of these such as CP55940 (Fig. 1) have provided valuable research tools for the study of the mechanisms of cannabinoid receptor activity. Sterling Research Institute discovered a novel class of aminoalkylindole analgesics that acted as agonists at the cannabinoid receptor in brain, with WIN55212-2 being the prototype (Compton et al., 1992; Pacheco et al., 1991) (Fig. 1). Structural analogs of this series have been investigated (Huffman, 1999). These first generation cannabinoid, non-classical cannabinoid and aminoalkylindole compounds exhibit limited, if any, selectivity for CB_1 versus CB_2 receptors.

1.2. Endocannabinoid compounds

The body's natural agonists for cannabinoid receptors are arachidonic acid metabolites (Fig. 1), including arachidonylethanolamide (anandamide), 2-arachidonoylglycerol (2-AG), and 2-arachidonylglycerylether (noladin ether) (see Di Marzo et al., 1999; Freund et al., 2003; Giuffrida et al., 2001; Howlett, 2002; Martin et al., 1999; Schmid, 2000; Sugiura and Waku, 2000 for review). Analogs of these eicosanoid "endocannabinoid" compounds have been synthesized in efforts to evade esterase and amidase metabolic breakdown, and increase specificity for the receptors rather than fatty acid amide hydrolases (FAAH) (Reggio and Traore, 2000).

1.3. Antagonists for cannabimimetic responses

The first specific antagonist to the CB_1 cannabinoid receptor was SR141716 (rimonabant) (Fig. 1), a compound discovered in a high throughput screening program at Sanofi Recherche (Rinaldi-Carmona et al., 1994; Barth and Rinaldi-Carmona, 1999). Because rimonabant can block dysfunctional craving for food and drugs (Duarte et al., 2004; Lallemand et al., 2001; Poncelet et al., 2003; Cohen et al., 2002; De Vries et al., 2001, 2003), it is currently undergoing clinical trials for obesity, smoking cessation and alcohol abuse (Black, 2004; Fernandez and Allison, 2004). A specific CB_2 receptor antagonist, SR144528 (Rinaldi-Carmona et al., 1998), may be clinically useful in immune modulation.

2. Localization of cannabinoid receptors

2.1. Radioreceptor ligand binding and in situ hybridizaton of CB_1 receptors

CB_1 receptors are among the most abundant G-protein-coupled receptors in brain, their densities being similar to levels of γ-aminobutyric acid (GABA)- and glutamate-gated ion channels. The distribution of cannabinoid receptors within the central nervous system was first described by Miles Herkenham in a landmark study using quantitative in vitro receptor autoradiography with the radioligand [^{3}H]CP55940 (Herkenham et al., 1991). The distribution of CB_1 receptors is highly heterogeneous with the highest densities of receptors present in the outflow nuclei of the basal ganglia, substantia nigra pars reticulata, and the internal and external segments of the globus pallidus. In addition, very high levels of binding are present in the hippocampus, particularly within the dentate gyrus, as well as in the molecular layer of the cerebellum. In contrast, there are few CB_1 receptors in the brainstem which may account for the lack of toxicity associated with very high doses of Δ^9-THC or other cannabinoid ligands.

There is a similar distribution of CB_1 receptors in humans (Glass et al., 1997; Biegon and Kerman, 2001). It is here, however, that the pattern of receptor localization within the cortex can be best ascertained within the more elaborate human cortex. The highest densities are found in association and limbic cortices, with much lower levels within primary sensory and motor regions, suggesting an important role in motivational (limbic) and cognitive (association) information processing.

Recent studies using immunohistochemical approaches with antibodies to either the C- (Egertova and Elphick, 2000) or N-terminal (Pettit et al., 1998; Tsou et al., 1998) of the CB_1 receptor and to FAAH, the enzyme proposed to catalyze the hydrolysis of endocannabinoids (Egertova et al., 1998), corroborate the earlier autoradiographic studies, thus substantiating the specificity of the ligands for the CB_1 receptor. These studies have also provided insight into the sub-cellular distribution of CB_1 receptors in areas such as hippocampus and amygdala (Katona et al., 1999, 2001). Combined with electron microscopy and electrophysiology studies, CB_1 receptors have been shown to be localized presynaptically on GABAergic interneurons (Katona et al., 1999, 2001). This would be consistent with the proposed role of endocannabinoid compounds in modulating neurotransmission. Hence, the anatomy of CB_1 receptors can provide clues to their function.

2.2. Functional mapping of CB_1 receptors

The distribution alone, however, cannot establish the functional significance of CB_1 receptors, nor how the actions of various cannabinoid ligands, both exogenous and endogenous, at these receptor sites translate to behavior. One approach to this question has been the use of neuroimaging methods to measure functional activity in the central nervous system (CNS). Given the widespread distribution of CB_1 receptors, and the variety of pharmacological, physiological and behavioral effects attributed to cannabinoid drugs, whole brain

neuroimaging methods are particularly well suited for identifying the functional role of these receptors.

In some of the earliest and simplest studies, it was established that the administration of Δ^9-THC and other cannabinoid ligands produced widespread dose dependent alterations in brain function in basal ganglia, hippocampus (Fig. 2), cerebellum, amygdala, and striatum (Goldman et al., 1975; Margulies and Hammer Jr., 1991; Bloom et al., 1997; Stein et al., 1998; Pontieri et al., 1999; Freedland et al., 2002). These changes parallel closely both the dose dependent nature of the effects on cannabinoid-induced behaviors and the time course of the onset of these behaviors, indicating that these alterations in functional activity are the substrates of these behaviors.

The behaviors and the changes in behaviors that result from the administration of cannabinoid drugs, particularly Δ^9-THC, can vary substantially in duration, with some disruptions in task performance observed as long as 24 h after Δ^9-THC exposure (Yesavage et al., 1985). Alterations in functional brain activity are also time dependent (Whitlow et al., 2002). Immediately following the administration of Δ^9-THC, for example, alterations in cerebral metabolism are widespread. However, by 6 and 24 h after administration, only a subset of these regions are affected, paralleling the time course of behavioral changes in locomotor activity. At these later time points, alterations in functional activity are found predominantly within portions of the limbic system (amygdala, ventral striatum, and prefrontal cortex). That functional activity remains altered in these areas suggests that behaviors subserved by these structures (e.g., anxiety, stress, and reward) may still be affected long after exposure to Δ^9-THC and may contribute to the abuse liability of Δ^9-THC.

Identifying the actions of the endocannabinoids at cannabinoid receptors has proved a more difficult problem, since their actions are very short-lived and therefore beyond the time resolution of many neuroimaging methods. One strategy employed by Stein and colleagues (Stein et al., 1998) involves the measurement of blood flow, which requires only a 1-min experimental period, following the administration of anandamide. These investigators showed altered blood flow in amygdala, hippocampus, striatum, and limbic cortex. These changes would be consistent with the role of endogenous cannabinoids in memory processes (Marsicano et al., 2002).

Another strategy has been to investigate the effects of the administration of the CB1 antagonist, rimonabant, thereby blocking endogenous cannabinoid tone in the brain. Although rimonabant blocks the effects of various exogenous cannabinoid agonists, it also evokes actions in the absence of any agonist, inhibiting many aspects of ingestive behavior, for example. This is particularly relevant given the link between hypothalamic endocannabinoids and regulation of body weight (Di Marzo et al., 2000). Rimonabant administered to animals responding for food produced a highly restricted pattern of changes in metabolic activity focused in the hypothalamus, nucleus accumbens, and extended amygdala, all areas central to the appetitive and consummatory aspects of feeding (Freedland et al., 2003). Here again, the pattern of changes in functional activity directly matches the behavioral profile. The close correspondence between the parameters of behavioral effects associated with the cannabinoid agonists, both exogenous and endogenous, provides an important tool for further studies of the actions of the CB1 receptor in the central nervous system.

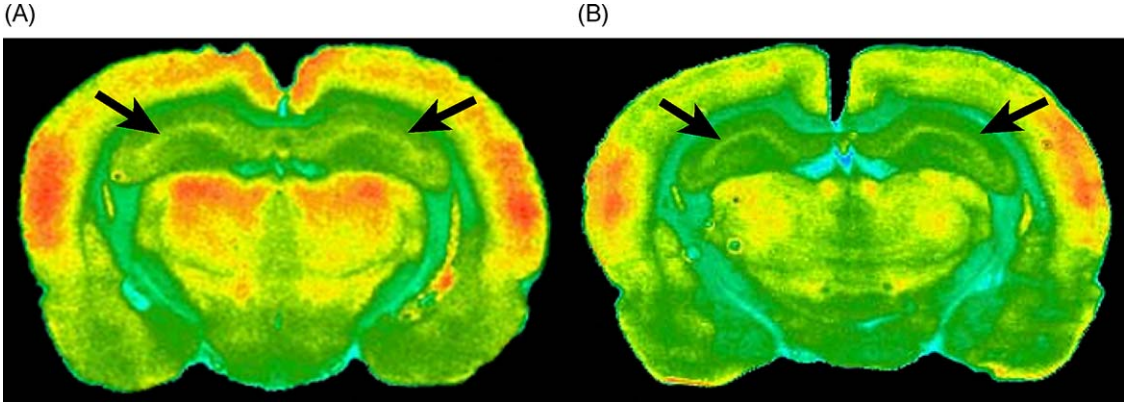

Fig. 2. Effects of the acute administration of Δ^9-THC on rates of glucose utilization in rats when the 2-[^{14}C]deoxyglucose method was applied 15 min after administration. Shown are color-coded transformations of autoradiograms of coronal sections of rat brain at the level of the hippocampus. Each color represents a range of rates of local cerebral glucose utilization (μmol/100 g/min). A is from an animal receiving vehicle (0.0 mg/kg Δ^9-THC) and B from an animal treated with 2.5 mg/kg Δ^9-THC. The arrows point to the hippocampus in each section.

2.3. Distribution of CB2 receptors

CB2 receptor mRNA has been found in spleen, tonsils and thymus, which are the major tissues of immune cell production and regulation (see Howlett et al., 2002; Cabral and Dove Pettit, 1998 for review). CB2 mRNA has been localized to B and T lymphocytes, natural killer cells, monocytes, macrophages and microglial cells, mast cells, as well as cultured cell models of these immune cells. CB2 agonists are generally suppressive of function of these cells, but both CB1 and CB2 receptors might contribute to these effects (Cabral and Dove Pettit, 1998).

3. Signal transduction mechanisms of cannabinoid receptors

Both CB1 and CB2 cannabinoid receptors are members of the superfamily of G-protein-coupled receptors, so the signal transduction properties of these receptors are mediated by the process of G-protein activation. The use of specific signal transduction assays was a critical turning point in the identification of specific cannabinoid receptors. After many years of speculation, the existence of cannabinoid receptors was confirmed when Howlett and colleagues showed that cannabinoids decreased cAMP in neuroblastoma cell cultures in a pertussis toxin-sensitive manner (Howlett, 1984), suggesting mediation by a $G_{i/o}$-coupled receptor (Howlett and Fleming, 1984; Howlett, 1985; Howlett et al., 1986).

3.1. G-protein-coupling

As observed with other G-protein-coupled receptors, guanine nucleotides inhibit cannabinoid agonist binding (Devane et al., 1988). Moreover, cannabinoid binding sites can be solubilized together with G-proteins from membranes (Houston and Howlett, 1993). Studies using a photoaffinity GTP analog (Prather et al., 2000) have shown that cannabinoid agonists activate multiple subtypes of $G_{i/o}$ α subunits with different potencies. Cannabinoid receptor activation of G-proteins in isolated membranes can be measured by agonist-stimulated [^{35}S]GTPγS binding (Burkey et al., 1997b; Selley et al., 1996), which follows the pharmacology of the brain cannabinoid receptor binding profile. In rat brain membranes, cannabinoid-stimulated [^{35}S]GTPγS binding is especially high because of the relatively large number of cannabinoid receptors in brain (Kuster et al., 1993). However, CB1 receptor coupling to G-proteins is relatively inefficient: in striatum, each cannabinoid receptor activates only three G-proteins, compared to 20 G-proteins for each mu and delta opioid receptor (Breivogel et al., 1997; Sim et al., 1996b). It is possible that this relatively low amplification is related to the high number of CB1 receptors: since these receptors exist in such high number in the brain, a high amplification between the receptor and transducer may not be necessary.

Agonist-stimulated [^{35}S]GTPγS binding can also be used to determine differences in agonist efficacy at the level of G-protein activation (Selley et al., 1997; Sim et al., 1996a). Using this technique in brain membranes, WIN55212-2 and levonantradol were full

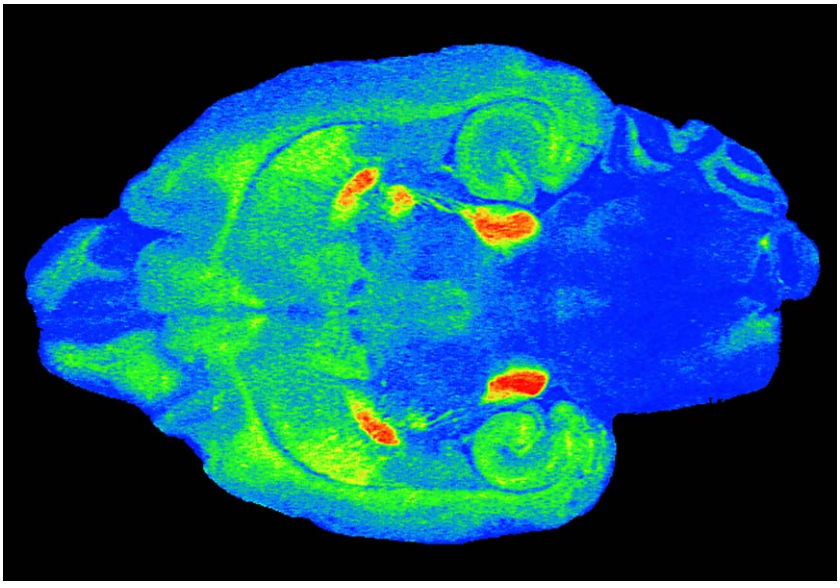

Fig. 3. [^{35}S]GTPγS autoradiography of cannabinoid-stimulated G-proteins in rat brain, using WIN55212-2 as an agonist. This horizontal section of rat brain shows high levels of activation in globus pallidus and substantia nigra, along with moderately high activation in hippocampus, cortex and cerebellum.

agonists, whereas anandamide produced partial efficacy (Breivogel et al., 1998) and Δ^9-THC was a weak partial agonist (Burkey et al., 1997b; Sim et al., 1996a). There is a discrepancy in the actions of the CB_1 antagonist rimonabant in [^{35}S]GTPγS experiments: in rat cerebellar membranes, rimonabant was a neutral antagonist, with no effect on [^{35}S]GTPγS binding except at concentrations 10,000 10,000 times greater than its affinity at CB_1 receptors (Breivogel et al., 1998). In contrast, in CB_1-transfected Chinese hamster ovary (CHO) cells, rimonabant behaved as an inverse agonist, producing relatively potent inhibition of basal [^{35}S]GTPγS binding (Landsman et al., 1997).

[^{35}S]GTPγS autoradiography has provided an effective method of exploring the brain regional distribution of CB1 $_r$eceptor-activated G-proteins (Sim et al., 1996a). In general, the distribution of cannabinoid-activated G-proteins in brain (Fig. 3) parallels that of CB1 $_r$eceptor binding and mRNA (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992), with high levels observed in motor areas like globus pallidus, caudate, cerebellum and substantia nigra, as well as other areas like hippocampus. Detailed examination of cannabinoid receptors and activated G-proteins in the cingulate cortex (Sim-Selley and Childers, 2002) reveals that differences in receptor/G-protein amplification can occur within a specific brain region.

3.2. Cannabinoid receptor-coupled effectors

Cannabinoid receptor activation of G-proteins influences multiple effector systems. Cannabinoid inhibition of adenylyl cyclase has been demonstrated in several cell types (Bayewitch et al., 1995; Felder et al., 1995; Howlett, 1984; Pacheco et al., 1993; Slipetz et al., 1995), and in brain membranes (Bidaut-Russell et al., 1990; Childers et al., 1993; Pacheco et al., 1991, 1994). In general terms, the pharmacology of cannabinoid-inhibited adenylyl cyclase matches that of cannabinoid receptor binding (Pacheco et al., 1991), including competitive antagonism by rimonabant (Felder et al., 1995; Rinaldi-Carmona et al., 1994). The finding (Pacheco et al., 1993) that responses of several $G_{i/o}$-coupled receptor agonists are non-additive with cannabinoids in inhibiting adenylyl cyclase, but additive at the level of G-proteins, suggests that cannabinoid receptors share common adenylyl cyclase catalytic units but different G-proteins with other receptors like adenosine A1 and GABAB receptors. In addition to inhibiting adenylyl cyclase, cannabinoids have been shown to stimulate cAMP accumulation in both globus pallidus slices (Maneuf and Brotchie, 1997) and striatal neurons (Glass and Felder, 1997), possibly via activation of Gs.

As with other receptors coupled to $G_{i/o}$ proteins, activation of CB1 receptors decreases Ca^{2+} conductance (Caulfield and Brown, 1992; Mackie et al., 1993; Mackie and Hille, 1992; Shen et al., 1996) and increases K^+ conductance (Mackie et al., 1995). These effects on Ca^{2+} channels (Mackie and Hille, 1992) and G-protein-coupled inwardly rectifying K^+ (GIRK) channels (Henry and Chavkin, 1995) are due to direct coupling by G-proteins and independent of cAMP. There may be important differences in CB_1- and CB_2-mediated effectors, since CB_2 receptors in transfected CHO cells had no effect on these ion currents despite their ability to inhibit adenylyl cyclase (Felder et al., 1995).

In addition to ion channels, other G-protein-coupled effectors have been associated with cannabinoid receptors; the most well known is the cannabinoid-induced release of arachidonic acid, observed in several cell culture systems and mediated both by phospholipase activity and G-proteins (Burstein et al., 1991, 1994; Shivachar et al., 1996). Cannabinoid drugs acting via both CB_1 (Bouaboula et al., 1995) and CB_2 (Bouaboula et al., 1996) receptors activate mitogen-activated protein (MAP) kinase and induce Krox-24 expression, presumably via activation of G-protein βγ subunits. These findings illustrate the basic concept that G-protein-coupled receptors may operate through several different effectors, and each effector system may be designed for specific purposes.

4. Functional consequences of cannabinoid receptor activation

Cannabinoid receptors are among the most ubiquitous neurotransmitter elements in the mammalian brain, as they are present in almost every brain region and on many different types of neurons (Moldrich and Wenger, 2000). The multiple consequences of cannabinoid receptor activation, i.e., a reduction in adenylyl cyclase, modulation of ion channels and reduction in intracellular Ca^{2+}, provide an important basis for control of multiple cellular signaling processes within the brain (Breivogel et al., 1999; Deadwyler et al., 1993; Twitchell et al., 1997). This has become more significant now that at least two endocannabinoids, anandamide and 2-arachidonoylglycerol (2AG), have been isolated from brain extracts (Devane et al., 1992; Mechoulam et al., 1995; Cadas et al., 1996; Stella et al., 1997; Sugiura et al., 1995). While not entirely unknown, those mechanisms that release endocannabinoids, and, as a consequence, activate cannabinoid receptors, have yet to be coupled to explicit behavioral events (van der Stelt and Di Marzo, 2003; Freund et al., 2003). The current state of knowledge lacks definitive data to delineate the conditions and mechanisms involved in the release of endocannabinoids. Given this current level of understanding it is now important to increase our knowledge of endocannabinoid system operation in awake, behaving animals.

4.1. Retrograde modification of synaptic processes by endocannabinoids

One of the most important breakthroughs in understanding how endocannabinoids produce functional changes in the CNS has come from the recent work of Wilson and Nicoll (Wilson et al., 2001; Wilson and Nicoll, 2001) linking cannabinoid receptors to a phenomena previously well characterized by Alger and colleagues (Alger et al., 1996; Alger, 2002; Pitler and Alger, 1994), known as depolarization-induced suppression of inhibition (DSI). The process of DSI entails suppression of GABA-mediated inhibition of hippocampal pyramidal cells as a function of the level and duration of pyramidal cell depolarization (Alger et al., 1996; Alger, 2002; Lenz and Alger, 1999; Varma et al., 2002). Wilson and Nicoll (2001) showed that DSI could be blocked by administration of the CB_1 receptor antagonist, SR141716A (rimonabant), which implicates release of endocannabinoids and participation of presynaptic CB_1 receptors in this process. The reduction in inhibition was further shown to involve a particular subpopulation of GABAergic interneurons in the hippocampus (Wilson et al., 2001). Carlson and colleagues (Carlson et al., 2002) recently reported that, if induced, this phenomenon could facilitate other forms of synaptic plasticity at different synapses on the same neurons.

In the hippocampus, DSI is initiated by depolarization-induced opening of N-type voltage-controlled Ca^{2+} channels (VCCs) (Wilson and Nicoll, 2002), which in turn effects release of endocannabinoids via a Ca^{2+} dependent process (Piomelli, 2003). Endocannabinoid compounds diffuse from the post-synaptic membrane and bind to CB_1 receptors on presynaptic terminals of a subclass of interneurons, where they inhibit release of GABA (Alger, 2002). The process by which diminished release of GABA occurs has not been thoroughly characterized, but may involve K^+ channels (Daniel et al., 2004). This retrograde, post-to-presynaptic linkage can serve as an elegant mechanism by which endocannabinoids regulate the excitability of hippocampal neurons.

4.2. What conditions release endocannabinoids in vivo?

Recently, we have tried to identify patterns of activity generated by hippocampal neurons in vivo during hippocampal dependent behaviors that could evoke release of endocannabinoids when tested in vitro (Hampson et al., 2003). However, we have failed to observe endocannabinoid release under these conditions due to a difference in the frequency of firing of hippocampal neurons in vivo versus what has been optimally demonstrated in vitro to produce DSI. As shown in Fig. 4, optimizing conditions for eliciting DSI in vitro via: (1) elevated intracellular free Ca^{2+} levels (20 μM), (2) increased activation of cholinergic receptors (carbachol, 3 μM) (Fig. 4A and C), and/or (3) simultaneous activation of metabotropic glutamate (mGluR1) receptors (with (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD)), was not sufficient with firing patterns generated in behavioral contexts and applied to the cell (Fig. 4D Hampson et al., 2002; Zhuang et al., 2003). Thus, the patterned frequency of depolarizing pulses generated by these cells under behavioral conditions was not sufficient to produce DSI (and therefore endocannabinoid release) when "played" into hippocampal cells under in vitro recording conditions.

When endocannabinoids are released, they act in a retrograde manner to decrease release of either inhibitory or excitatory transmitters (Alger et al., 1996). This is a CB_1 receptor-mediated process but only occurs under conditions in which intracellular Ca^{2+} levels increase above resting (Piomelli, 2003). By examining hippocampal cell firing in vivo we have determined that such occurrences for a single neuron are relatively rare, but the probability of endocannabinoid release could be increased by the convergence of other synchronous synaptic events (Kim et al., 2002; Varma et al., 2001). Endocannabinoid regulation of synaptic transmission in hippocampus occurs only when there is elevated Ca^{2+} in the same cell that exhibits DSI. However, in the cerebellum, endocannabinoid influence on excitatory synaptic transmission may not be a Ca^{2+}-mediated event (Daniel et al., 2004).

What then is the functional significance of this endocannabinoid dependent suppressed inhibition, if it only occurs under very rare circumstances in hippocampal neurons? One possibility is to facilitate VCC conductances, thereby modulating intracellular Ca^{2+} levels during bursts of action potentials in the post-synaptic neuron. Because DSI has a fairly long time constant (10 s), such prolonged reduction in GABAergic inhibition to the cell would facilitate burst occurrences and VCC conductance changes over that time frame (Fig. 4A). This could initiate a positive feedback in which high frequency discharges by the neuron in the DSI "window" would become more likely to depolarize the membrane to activate VCCs and elevate intracellular Ca^{2+} levels, in turn further reducing inhibitory inputs to the cell via increased Ca^{2+} dependent release of endocannabinoids. Such a positive feedback process would, however, be highly dependent upon sustaining the elevation in frequency and efficacy of excitatory inputs to the post-synaptic neuron (Hampson et al., 2003; Zhuang et al., 2003; Alger et al., 1996; Beau and Alger, 1998; Morishita et al., 1998). One possible role for endocannabinoids via the DSI mechanism would therefore be to provide a means of sculpting out patterns of activation produced by multiple inputs to restricted "patches" of neurons within the tightly

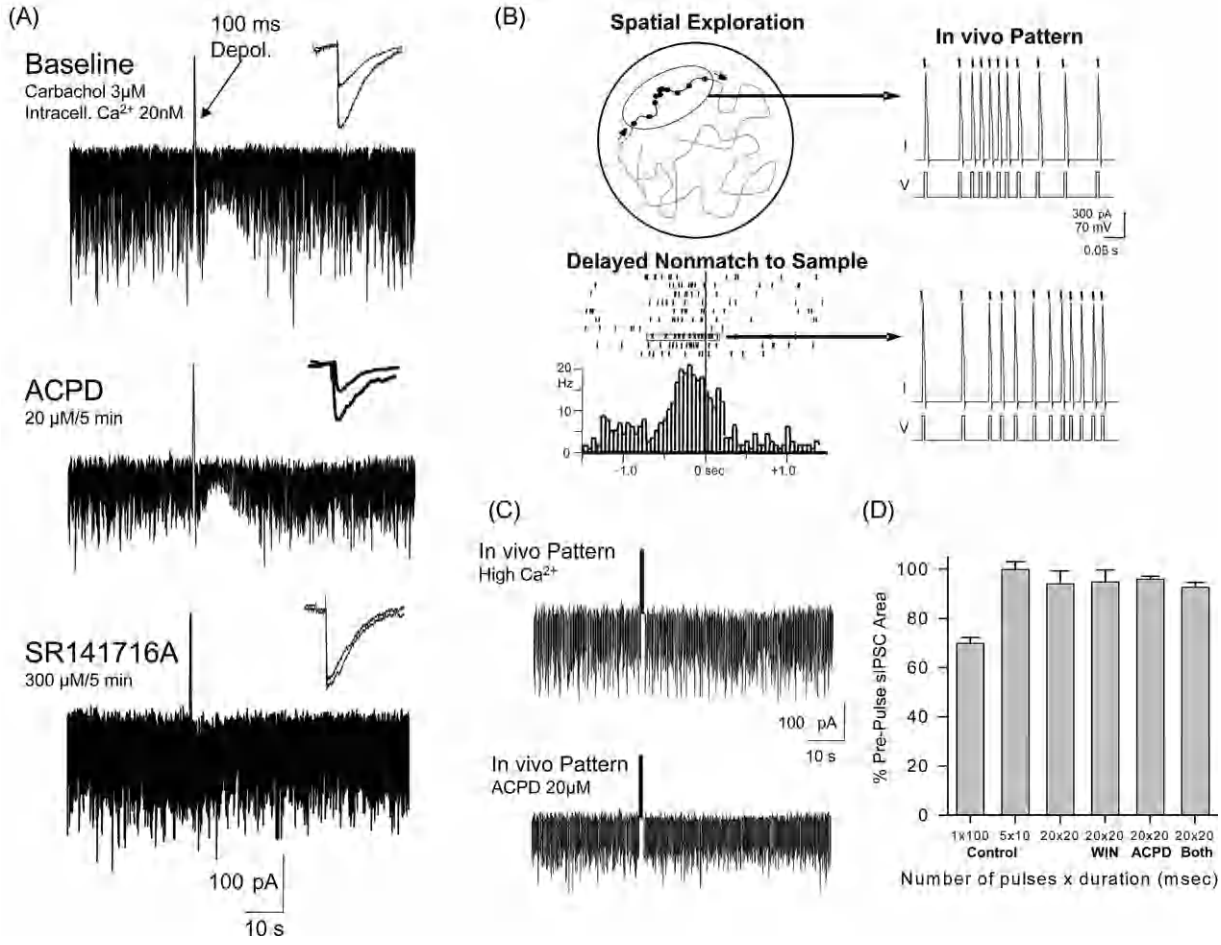

Fig. 4. Hippocampal pyramidal cell recordings from in vitro slices. (A) DSI shown as reduction in spontaneous inhibitory post-synaptic currents (IPSCs) (inverted due to KCl in recording pipette) induced by a 100 ms depolarizing voltage step (0 mV) delivered to hippocampal pyramidal cell under voltage clamp and optimal bath conditions (carbachol 3 μM , intracellular Ca^{2+} 20 μM with low buffering; Top) and with mGluR1 receptor activation (ACPD 20 μM, Middle). DSI is blocked by the addition of the CB_1 receptor antagonist rimonabant (100 nM, Bottom). Insets show difference in amplitude and area of Schaeffer collateral stimulus evoked IPSPs before and after the depolarizing pulse. (B) Examples of behaviorally relevant firing patterns of hippocampal pyramidal cells recorded under indicated testing paradigms used to program post-synaptic depolarization patterns in vitro. Right: In vivo hippocampal firing patterns are simulated by trains of 1.0 ms duration action potential-like pulses at different frequencies that did not produce DSI under the same recording conditions as in A. (C) Lack of DSI (see A) induction by in vivo firing patterns under high carbachol, and Ca^{2+} or ACPD recording conditions. (D) The graph shows that a 100 ms depolarizing pulse (1 × 100) was the minimal condition for producing DSI. This could not be duplicated by stimulus patterns that mimicked the in vivo hippocampal firing. Mean ± SEM over 12-0 cells each.

packed hippocampal cell layers. Since the distance of effective diffusion of endocannabinoid molecules from a given neuronal release site appears limited to only 20-40 microns (Wilson et al., 2001), the above process would target only adjacent neurons for modification via common inputs (Alger, 2002).

We have been able to show that only a very high frequency range of firing (>30 Hz) produces endocannabinoid release, and that this type of firing in hippocampal neurons is very infrequent in vivo (Deadwyler and Hampson, 2004; Hampson et al., 1999, 2001, 2002). When hippocampal neurons satisfy this high frequency firing requirement is when they fire "bursts" of action potentials (Zhuang et al., 2003). However, such burst-type firing is not foreign to hippocampal neurons, in fact it reflects the hallmark of a hyperexcitable state in these neurons such as epileptic discharges, a condition that produces DSI (Beau and Alger, 1998). It can be further surmised that slow wave sleep (Csicsvari et al., 2000; Buzsaki, 1998), in which hippocampal cell burst firing is more likely, is also a potential occasion for endocannabinoid release. The basis of endocannabinoid release and therefore its true function in hippocampus requires that this process be identified under normal operational conditions in hippocampal neurons. Once identified, verification will necessarily involve the disruption of that same behavioral process by application of CB_1 receptor antagonists. Given the

recent advances in detection of these retrograde mediators, and a greater understanding of the behaviors that endocannabinoid compounds modulate, it would not be surprising to find that both of these criteria are satisfied in the near future.

5. Chronic cannabinoid effects on receptors and signal transduction systems

Chronic administration of cannabinoid drugs to animals results in tolerance to many of the acute effects of Δ^9-THC, including memory disruption (Deadwyler et al., 1995), decreased locomotion (Abood et al., 1993; Oviedo et al., 1993), hypothermia (Fan et al., 1996; Pertwee et al., 1993), neuroendocrine effects (Rodriguez de Fonseca et al., 1991), and analgesia (Adams and Martin, 1996). Both cannabinoid receptors and their signal transduction systems are significantly regulated by chronic agonist exposure; these effects have been recently reviewed in detail (Sim-Selley, 2004).

Brain cannabinoid receptor levels usually decrease after prolonged exposure to agonists (Fan et al., 1996; Oviedo et al., 1993; Rodriguez de Fonseca et al., 1994; Romero et al., 1997), although some studies have reported increases (Romero et al., 1995) or no changes (Abood et al., 1993) in receptor binding in brain. Appropriate controls have demonstrated that down-regulation of cannabinoid receptors is homologous. Differences among studies may depend on the treatment agonist used, brain region examined or treatment time. For example, levonantradol produces a greater desensitization of adenylyl cyclase inhibition than Δ^9-THC in cultured neuroblastoma cells (Dill and Howlett, 1988), which may be explained by the efficacy differences between these two agonists (Burkey et al., 1997a; Sim et al., 1996a). Furthermore, a time course study revealed differences in the rates and magnitudes of receptor down-regulation across brain regions (Breivogel et al., 1999). These findings suggest that tolerance may develop at different rates to different effects of cannabinoids.

Chronic treatment with Δ^9-THC also produces variable effects on cannabinoid-mediated signal transduction systems. For example, one study examining the effect of in vivo chronic CP55940 treatments found no change in adenylyl cyclase in cerebellar membranes despite a 50% reduction in receptor binding (Fan et al., 1996), suggesting the presence of receptor reserve. Chronic Δ^9-THC treatment produces significant desensitization of cannabinoid-activated G-proteins in a number of rat brain regions, as determined by cannabinoid-stimulated [^{35}S]GTPγS autoradiography (Sim et al., 1996a). Moreover, the time course of the decrease in cannabinoid-stimulated [^{35}S]GTPγS binding varied across brain regions (Breivogel et al., 1999).

6. The next 30 years of cannabinoid physiology and pharmacology

Cannabinoid receptor agonists were developed by the pharmaceutical industry as non-NSAID, non-opioid analgesics; however, their therapeutic utility was curtailed due to untoward side effects such as sedation and cognitive dysfunction (Johnson et al., 1981). Both beneficial and untoward effects were believed to be the result of CB_1 receptor activation. As the result of landmark studies in CB_1 −/− knock-out mice (Di Marzo et al., 2000; Breivogel et al., 2001), we now understand that the endocannabinoid anandamide and the aminoalkylindole WIN55212-2 exhibit antinociceptive properties and activate G-proteins in brain in the absence of CB_1 receptors. The pharmacology differs from that of the CB_1 receptor in that classical and non-classical CB_1 agonists failed to evoke in vivo behaviors and in vitro regulation of G-proteins, and the responses were not antagonized by rimonabant (Di Marzo et al., 2000; Breivogel et al., 2001). Antinociception in inflammatory pain models was elicited by palmitoylethanolamide and antagonized by the CB_2 receptor antagonist SR144528, suggesting the involvement of a "CB_2-like" receptor (Calignano et al., 2001). Studies of cardiovascular function have elucidated a novel endothelial cell G-protein-coupled receptor that regulates vasorelaxation in response to anandamide, methanandamide and abnormal cannabidiol, but not cannabinoid or aminoalkylindole compounds (Jarai et al., 1999; Wagner et al., 1999; Mukhopadhyay et al., 2002). Rimonabant was a poorly potent inhibitor, but the cannabidiol analog O-1918 served as a relatively selective antagonist (Offertaler et al., 2003). Anandamide could also promote vasorelaxation via the VR1 vanilloid receptor (Zygmunt et al., 1999). These studies open up the potential for drug development aimed at potentially novel receptors, and allow for the possibility of drug discovery for multiple therapeutic purposes.

Studies with CB_1 antagonists have demonstrated the importance of cannabinoid receptors in the phenomenon of craving. Craving has been the single most difficult obstacle to the effective treatment of substance abuse disorders. Rimonabant has been successful in clinical trials of weight loss because it ameliorates the pathologically heightened salience of food (Black, 2004). Similarly, rimonabant is being found to be an effective adjunct to behavior modification in smoking cessation (Fernandez and Allison, 2004). Studies in rodent models of appetitive behaviors for reinforcing drugs have indicated that this CB_1 antagonist reduces the self-administration of cocaine and opioids (De Vries et al., 2001, 2003). It can be predicted that our new appreciation of the role of the cannabinoid receptor systems in reinforcement will result in greater

research in reinforcing stimuli. It can be predicted that in the coming years, the clinical utility of CB_1 antagonists will surpass the use of long-acting opioid receptor agonists and partial agonists as the hallmark treatment for substance abuse disorders.

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